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Soil animals and nitrogen mineralization under sand-fixation plantations in Zhanggutai region, China

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Abstract: The effects of soil animals on soil nitrogen (N) mineralization and its availability were studied by investigating soil animal groups and their amounts of macro-faunas sorted by hand, and middle and microfaunas distinguished with Tullgren and Baermann methods under three *Pinus sylvestris* var. *mongolica* Litv. plantations in Zhanggutai sandy land, China. In addition, soil N mineralization rate was also measured with PVC closed-top tube *in situ* incubation method. The soil animals collected during growing season belonged to 13 orders, 5 groups, 4 phyla, whose average density was 86 249.17 individuals·m^{-2}. There were significant differences in soil animal species, densities, diversities and evenness among three plantations. Permanent grazing resulted in decrease of soil animal species and diversity. The average ammonification, nitrification and mineralization rates were 0.48 g·m^{-2}·a^{-1}, 3.68 g·m^{-2}·a^{-1} and 4.16 g·m^{-2}·a^{-1}, respectively. The ammonification rate in near-mature forest was higher than that in middle-age forests, while the order of nitrification and net mineralization rates was: middle-age forest without grazing < middle-age forest with grazing < near-mature forest with grazing (P<0.05). Soil N mineralization rate increased with soil animal amounts, but no significant relationship with diversity. The contribution of soil animals to N mineralization was different for different ecosystems due to influences of complex factors including grazing, soil characteristics, the quality and amount of litter on N mineralization.

Keywords: Soil animals; N mineralization; *Pinus sylvestris* var. *mongolica* Litv.; Zhanggutai sandy land

Introduction

The role of biodiversity on ecosystem function is one of the subjects at the forefront of ecology (Schulze & Mooney 1994; Naeem *et al*. 1994; Tilman *et al*. 1996, 1997; Lawton *et al*. 1998). Many researchers found that terrestrial ecosystem functions primarily depend on soil organism diversity, and thus they suggested that many ecological issues should first consider underground processes (Alphei *et al*. 1996; Heneghan *et al*. 1999; Copley 2000). Soil animals are an extremely diverse group of organisms in soil ecosystem and most of invertebrate organisms live in the soil in most of their life. Soil biodiversity and its absolute amounts are very high, and there might be more than 10 thousand arthropods and millions of nematodes in a 1-m^2 plot. Meanwhile, soil animal biomass in the global soils might as much as 600 hundred million people (Yin 2000). Therefore, soil animals were considered as one of the most important pools of matters and energy in terrestrial ecosystems.

Soil animals perform several functions in soils, which make them a key part of all terrestrial ecosystems. In general, the role of soil microbes in decomposition of organic matter and mineralization of nutrients were emphasized; however, the ecosystem functions of soil animals were neglected. Soil animals act as 'ecosystem engineers', whose ecological functions include litter fragmentation, micro-environment creation for reproduction of other organisms, soil physio-chemical characteistics alteration, and direct transformation of organic matters into inorganic nutrients which are directly absorbed by plants (Yin 2000).

Nitrogen (N) is generally one of the key elements, which limit plant growth in many terrestrial ecosystems (Chen *et al*. 2006). Nitrogen cycling involves many kinds of biological processes. In most ecosystems, N mineralization rate, which is primarily controlled by soil organisms, determines the N availability for plant growth. Recently, more and more researchers have recognized the important role of soil animals in soil N cycling. On the one hand, soil animals have the dominant role in soil food chains, and occupy a higher trophic level. On the other hand, soil animals have very high biodiversity and huge biomass. Finally, the seasonal dynamics of soil animals is advantageous to regulate the N cycling. Soil animals can increase soil N mineralization rate to satisfy plant needs for N through enhancing their activities during growing seasons, and decrease soil N mineralization rate to avoid N losses through decelerating their activities. Currently, some reports about the influences of earthworms, nematodes and termites on N availability have been published. Seastedt (1984) reported that inoculation of springtail or multiped can improve soil N content (2–4 times NH_4^+-N and NO_3^+-N) in forest

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ecosystems. Heneghan *et al*. (1999) found that nematodes can control N minerailization rate.

To combat desertification, prevent soil erosion and decrease sandy storm frequency and intensity, Mongolian pine (*Pinus sylvestris* var. *mongolica* Litv.) was first introduced as an afforestation species to Zhanggutai sandy land (42°43´N, 122°22´E), the southeastern border of Keerqin sandy land in 1955. Because of its good ecological adaptation at early growth stage, this tree species has also been widely used as a good afforestation species for stabilizing sandy dunes in the northern area of China (Chen *et al*. 2002). Although there currently are many studies on the physiological and ecological characteristics of Mongolian pine, soil nutrients cycling and ecosystem stability under Mongolian pine plantations (Zeng *et al*. 1996; Chen *et al*. 2002; Jiang *et al*. 2002; Zhu *et al*. 2005; Chen *et al*. 2006), few attentions are paid to how afforestation of Mongolian pine affects soil animal biodiversity and its distribution in the soil. The objectives of this study, therefore, are: 1) to investigate soil animal species numbers, diversity and density; 2) to explore the relationship between soil animal biodiversity and N mineralization rate.

Materials and methods

Study site

The experimental forests were located in the southeastern part of the Keerqin Sand Land (42°43' N, 122°22' E, and 226.5 m altitude). Historically, this area was a typical ecotone of forest and pasture, but now it becomes a mainly agricultural and pasture region with degraded vegetation induced by overcultivation and overgrazing (Jiang *et al*. 2002). The area is a semi-arid region with sandy soils, low precipitation (450 mm per year), and high potential evaporation (1 300–1 800 mm per year). The average annual temperature is 6.2ºC; the relative air humidity is 59%; and the average annual frost-free period is 150 days. Soils have developed on wind-deposited sands and are characterized by coarse texture and loose structure with greater proportion of sands; thereby, essential soil nutrient (C, N, and P) contents are rather low (Chen *et al*. 2002). Herbages are the dominant vegetation in this area, which include Polygonaceae, Chenopodiaceae, Rosaceae, Fabaceae, Compositae, Graminaea, and Cyperaceae.

Sampling

In October 2003, three plots were established within the selected Mongolian pine stands with similar topography. Each plot area was 30 m × 20 m. Because of budgetary limitations, we had only one plot per treatment. The basic stand characteristics of the experimental Mongolian pine plantations, such as tree density, mean diameter at breast height (DBH), mean tree height, and so on, were described in Table 1, and the soil bulk density, pH value and nutrient concentrations of the experimental plots were described in Table 2. The three plantations could be classified into middle-age stand (30-year-old: ZSM30 and ZSL30) and near-mature stand (47-year-old plantation: ZSL47), according to the criterion by Jiang *et al*. (2002).

Table 1. Characteristics of the experimental Mongolian pine plantations

Stands	Age (a)	Density (trees·hm^{-2})	Diameter* (cm)	Tree height* (m)	Forest floor biomass (kg·hm^{-2})	Management practices
ZSM30	30	850	14.35±0.80a	8.01±0.20a	8000	Non-grazing
ZSL30	30	825	14.15±0.63a	7.93±0.13a	3500	Free-grazing
ZSL47	47	725	19.28±1.98b	13.46±0.86b	5000	Free-grazing

Notes: *Mean ± 1 SE, n >30; Values suffixed with same letters in the each column are not significant at $P < 0.05$ level.

Table 2. Basic soil characters (0-15 cm) in three Mongolian pine plantations

Stands	Bulk density (g·cm^{-3})	pH	Organic carbon (g·kg^{-1})	Total nitrogen (g·kg^{-1})	Total phosphorus (g·kg^{-1})	C/N	N/P
ZSM30	1.62±0.01a	6.37±0.07a	3.90±0.14a	0.395±0.038a	0.101±0.007a	9.87±1.94a	3.91±0.98a
ZSL30	1.65±0.01a	6.67±0.05a	3.97±0.08a	0.415±0.006a	0.111±0.002a	9.57±2.17a	3.74±0.98a
ZSL47	1.67±0.03b	6.09±0.11b	7.37±0.50b	0.533±0.008b	0.141±0.001a	13.83±3.65b	3.78±0.76a

Notes: *Mean ± 1 SE, n=6; Values suffixed with same letters in the each column are not significant at $P < 0.05$ level.

Experimental design and methods

In July 2004, bigger soil animals were collected by hand with a large sample core of 50 cm × 50 cm. Soil samples for mesofauna and small groups (microarthropolds, nematodes and echvtraeids) were collected with a metal cylinder in volume (10 cm × 10 cm in diameter × depth) and extracted through Tullgren funnels for 48 h (dry funnel method). Soil samples for wet animals were taken with a smaller metal cylinder in volume (5 cm × 5 cm in diameter × depth), wrapped in nylon cloth and extracted through Bearmann funnels for 48 h (wet funnel method) (Workgroup of Research Methods of Soil Fauna 1998).

Net N mineralization and net nitrification were estimated using the *in situ* closed-top core incubation method (Binkley *et al.* 1989) from October 16, 2003 to October 16, 2004. Near the center of each subplot, the forest floor was carefully removed and a top-caped polyvinyl chloride (PVC) tube with diameter of 4.0 cm and length of 20 cm was inserted into the soil for six field incubation cycles, each of which spans 1 month except for the first time, and the top 15-cm soils in the PVC tubes were collected as samples. During the first sampling time (from October 16, 2003 to April 16, 2004), soil was frost due to low temperature, and replacement of PVC tubes was very difficult. At the beginning of

each cycle, a pre-incubation soil was sampled near each tube to estimate the initial values of NH_4^+-N and NO_3^--N. A 30-g subsample from each of pre- or post-incubation soil samples was extracted with 100 mL 2 mol·L^{-1} KCl, shaken for 30 min, and kept overnight at 4°C in a refrigerator. The NH_4^+-N and NO_3^--N concentrations in the extracted solutions of each sample were separately measured with a spectrophotometry by the indophenol blue method and the cadmium reduction method (Liu *et al*. 1996).

For the background of soil physio-chemical properties in the three plots, the pre-incubation soil samples were used to measure soil bulk density, pH, organic matter, total N, and total P. Furthermore, 10-g soil from each of pre- or post-incubation soil samples was dried in an air-forced oven at 105°C to a constant weight for determining soil moisture. In addition, a soil sample was collected within each subplot by using a 5.0-cm-height sampling cylinder for determining soil bulk density at each of three soil layers: 0–5 cm, 5–10 cm, and 10–15 cm in April 2004. The soil bulk density (g·cm^{-3}) was determined based on the dry soil weight per unit volume of the soil core at each layer. Soil pH was measured in a 1:2.5 (soil: water) solution and deionized water using a glass electrode. The soil organic matter was determined by dichromate oxidation and titration with ferrous ammonium sulphate. Total N was determined by the microkjeldahl digestion method (Liu *et al*. 1996).

Data processing

Soil animal diversity index was calculated by Shannon biodiversity index:

$$H' = -\sum_{i=1}^{s} P_i \ln P_i$$

where H' is the biodiversity index, s the total soil animal groups, and P_i is the proportion of group i among s groups.

The evenness of soil animal groups (e) is as follows,

$$e = H' / \ln s$$

The net N mineralization (mg·kg^{-1}) is the net changes in NH_4^+-N and NO_3^--N and the net nitrification (mg·kg^{-1}) is the net changes in NO_3^--N relative to their initial values in the incubation. Annual net N mineralization was estimated as the sum of the net NH_4^+-N and NO_3^--N that were produced over the growing season. The conversion of annual net N mineralization unit from mg N per kg dry soil to g·N·m^{-1} was based on the bulk density at 15-cm soil in each plot. Inorganic N was calculated by NH_4^+-N plus NO_3^--N from pre-incubation soil samples. Soil animal diversity index was calculated with software Bio-DAP and other data were analyzed with SPSS (10.0) (2001).

Results and discussion

The soil animals, collected during growing season, belonged to 13 orders, 5 groups, 4 phyla (Table 3), and average density was 86 249.17 individuals·m^{-2} under three Mongolian pine plantations in Zhanggutai sandy land. Nematoda was 76 310.67 individuals·m^{-2}, and accounted for 85.48% of all soil animals. In addition, there was an apparent difference among three plantations. Non-grazing (4 years prevention from grazing) plantation (ZSM30) had 13 orders and 5 groups of soil animals, and the animal density was 72 585.00 individuals·m^{-2}, which was a plantation that had most abundant soil animals and the lowest animal density compared with the grazing plantation (ZSL47). The density of dominant groups (Nematoda and Eutardigrada) was 69 205.00 individuals·m^{-2}, and accounted for 95.34% of all soil animals in this plantation. Soil animals belonged to 11 orders and 5 groups, and the density was 90 860.00 individuals·m^{-2} in the ZSL30 plantation, which was in the middle of the three pine plantations. The density of dominant groups (Nematoda and Eutardigrada) was 84 845.00 individuals·m^{-2}, which accounted for 93.35% of all soil animals in ZSL30. The density of Hymenoptera (ant) was also very high, which was a little less than the densities of Nematoda and Eutardigrada. In ZSL47, the soil animals belonged to 9 orders, 5 groups, which is the least of three plantations, while the density of soil animals (95 302.50 individuals·m^{-2}) was the highest among the three plantations. The density of Hymenoptera was 1 582.50 individuals·m^{-2}, which was less than the numbers of the dominant group (Nematoda, 90 202.50 individuals·m^{-2}, which accounted for 94.65% of all soil animals). The order of the soil animals diversity represented by either Shannon biodiversity index (H') or the evenness of soil animal groups (e) showed the same pattern: ZSM30> ZSL30 > ZSL47 (P<0.05) (Table 4).

Table 3. Soil animal components in three Mongolian pine plantations in Zhanggutai sandy land

Species	ZSM30		ZSL30		ZSL47	
	Density (ind·m^{-2})	Percent (%)	Density (ind·m^{-2})	Percent (%)	Density (ind·m^{-2})	Percent (%)
1. Nemathelminthes						
Nematoda	59485.00	81.95	79245.00	87.22	90202.50	94.65
2. Tardigrads						
Eutardigrada						
Eutardigrada	5222.50	7.20	5600.00	6.16	400.00	0.42
3. Annelida						
Oligochaeta						
Oligchacta-plesiopora	1032.50	1.42	452.50	0.50	852.50	0.89
4. Arthropoda						
Arachnida						
Araneae	800.00	1.10	567.50	0.62	240.00	0.25
Acarina	3697.50	5.09	75.00	0.08		
Insect						
Collembola	15.00	0.02	15.00	0.02		
Psocoptera	80.00	0.11			80.00	0.08
Orthoptera	560.00	0.77	160.00	0.18	480.00	0.50
Homoptera	80.00	0.11			400.00	0.42
Hemiptera	190.00	0.26	87.50	0.10		
Coleoptera	495.00	0.68	1215.00	1.34	575.00	0.60
Lepidoptera	87.50	0.12	80.00	0.09		
Diptera	120.00	0.17	882.50	0.97	490.00	0.51
Hymenoptera	720.00	0.99	2480.00	2.73	1582.50	1.66
Total	72585.00	100.00	90860.00	100.00	95302.50	100.00

Ammonification rates under ZSM30 and ZSL30 were not significantly different; however they were significantly lower than that under ZSL47 (P<0.05). Both nitrification and net N-mineralization rates showed the following order: ZSM30< ZSL47 < ZSL30 (P<0.05) (Table 5).

Combined with the results of soil animals and N-mineralization in three pine stands, the numbers, diversity and evenness of soil animal groups in ZSM30 were the highest, while soil ammonification, nitrification and net N-mineralization rate were the lowest. The numbers, diversity and evenness of soil animal groups in ZSL47 were the lowest, while soil ammonification, nitrification and net N-mineralization rates were the highest. All of these indicated that there were no positive relationship between soil animal diversity and N mineralization rate. However, there might be a positive relationship between the density of soil animal and N mineralization rate because the soil nitrification and net N-mineralization rate increased with the densities of soil animals (Tables 3 and 5).

Table 4. Soil animal diversity indexes and their *t*-test analysis in three Mongolian pine plantations in Zhanggutai sandy land

Stands	ZSM30	ZSL30	ZSL47
Shannon diversity index	0.78	0.58	0.31
Evenness	0.30	0.23	0.13
Difference（*t*-test）			
ZSM30	-		
ZSL30	(31.648, 149539)*	-	
ZSL47	(77.355, 139661)*	(49.700, 182050)*	-

Notes: *Significant difference at $P < 0.05$ level (*t* value, degree of freedom).

Table 5. Soil ammonification, nitrification and net N-mineralization rate in three Mongolian pine plantations in Zhanggutai sandy land ($g \cdot m^{-2} \cdot a^{-1}$)

Stands	Ammonification rate	Nitrification rate	Net N-mineralization rate
ZSM30	0.43±0.08a*	2.04±0.31a	2.46±0.36a
ZSL30	0.43±0.08a	4.19±0.37b	4.61±0.39b
ZSL47	0.59±0.11b	4.81±0.43c	5.40±0.47c

Notes: *Mean ± 1 SE, n=12; Values suffixed with same letters in the each column are not significant at $P < 0.05$ level.

We could not use statistics to make correlation analysis due to few research plots in this study. Similar situations were also occurred in many previous studies of other researchers (Seastedt 1984; Griffiths 1994; Schulze *et al.* 1994). However, we used the *t*-test analysis (n=12) to compare N mineralization rate per soil animal density (10 000 individuals$\cdot m^{-2}$) in three pine forests (data from Tables 3 and 5).

Previous studies had seldom made correlation analysis between soil animal diversity and N transformation process, which might mostly due to laborious work involved in the field experiments. However, several reports on the relationships between soil animals and nutrients were found (Haimi *et al.* 1992; Alphei *et al*. 1996). Faber *et al*. (1991) reported that soil N mineralization rate increased with the complexity of soil animal community, and addition of a new trophic level to the existing animal trophic levels was always advantageous to N mineralization and plant growth. Several other studies suggested that the influence of soil animal species diversity on N mineralization rate was not important under soil ecosystem, and it was different from the influence of aboveground organisms on ecosystem processes (Mikola *et al*. 1998). There was usually a weak correlation between soil animal diversity and N mineralization because most of soil animals were polyphagia, for example, earthworm and *Cognettia.* Thus, soil animals that had many trophic levels could perform complete ecosystem functions even when the species diversity of soil animals were very low trophic levels while even showed low species diversity (Walter 1987). Few studies also paid attention to the key species when they discussed the relationship between soil animals and N minerailzation. For example, *Cognettia sphagnetorum* could perform stronger ecosystem functions than several species of arthropod community. Sulkava *et al*. (1996) found that in a coniferous forest in northern Europe, *Cognettia sphagnetorum* had more influences on N mineralization than a typical small arthropod community, which further proved the importance of key species to ecosystem functions. In this study, we also found that key soil animal species might be more important than other species (data from Tables 3 and 5). The influence of soil animal group diversity on N mineralization was very weak (date from Tables 4 and 5), while soil animal density had a significant influence on the N mineralization (Tables 3 and 5). Thus, we proposed that Nematoda was the key functional group to N mineraliztion according to the same order of Nematoda density and net-N mineralization rate under three plots in our study site (Tables 3 and 5).

Further analysis on relationship between soil animal density and net-N mineralization rate showed the contribution rates of soil animals to net-N mineralization (i.e. net-N mineralization rate relative to 10 000 soil animal individuals) (g·10 000 individuals$^{-1}\cdot a^{-1}$) in ZSM30, ZSL30 and ZSL47 were 0.41, 0 .58 and 0.60 g·10 000 individuals$^{-1}\cdot a^{-1}$, respectively. The contribution of soil animals to net-N mineralization in ZSL30 and ZSL47 were no significant difference, while they were significantly higher than that in ZSM30 (P<0.05, n=12) (Tables 4 and 5). That is to say, the contribution rates of soil animals to net-N mineralization in both of grazing pine forests were similar, but higher than those in non-grazing forests. Combined with net-N mineralization rates in three pine forests, we deduced that soil animal diversity in non-grazing pine forest (ZSM30) increased with the quantity of leaf litter and the soil animal groups was no direct relationship with soil net-N mineralization rate. Although the increase of soil animals groups in non-grazing pine forest might increase soil N mineralization to an extent, however, soil microbe might immobilize more inorganic N with the accumulation of litterfall, thus led to a decrease in net-N mineralization rate. Some studies also showed there were different contribution rates of soil animals to net-N mineralization in different ecosystems. Griffiths (1994) reported that only 30% of N mineralization rate might be induced by soil animals, while Smith (1992) found that 88% of N mineralization rate was attributed to soil animals at the island of Marion. In this study, we also found that the contribution rates of soil animals to net-N mineralization in different ecosystems were variable. In addition, we also found that N mineralization rate might be influenced by many factors, including grazing, the quality and quantity of litter, soil physio-chemical properties, and soil animal density.

Conclusions

The soil animals, collected during growing season, belonged to 13 orders, 5 groups, 4 phyla, and average density was 86 249.17 individuals$\cdot m^{-2}$. Nematoda was 76 310.67 individuals$\cdot m^{-2}$, accounted for 85.48% of all soil animals. There were differences in soil animal species, densities, diversities and evenness among

three plantations, and permanent grazing resulted in decrease of soil animal species and diversity. Soil animal density was influenced by soil chemical and physical properties and quality and quantity of litter.

The average ammonification, nitrification and mineralization rate were 0.48 $g \cdot m^{-2} \cdot a^{-1}$, 3.68 $g \cdot m^{-2} \cdot a^{-1}$ and 4.16 $g \cdot m^{-2} \cdot a^{-1}$, respectively. The ammonification rate in near-mature forest was higher than those in middle-age forests, whereas the rank of nitrification and net mineralization rates was: middle-age forest without grazing < middle-age forest with grazing < near-mature forest with grazing ($P<0.05$). Soil N mineralization rate increased with the numbers of soil animals, but not with diversity. The contribution of soil animals on N mineralization was different according to ecosystem properties due to influence of complex factors on N mineralization.

By now, most of studies on soil animal and N mineralization are just in simulation research phase, and few are *in situ* field studies. Thus, the influence of soil animal on N mineralization needs to be further studied. The assessment of ecosystem functions of soil animal and its relationship with N mineralization are important subjects in ecosystem ecology.

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